SUMMARY OF COMMENTS - SYMPOSIUM ON METHODS FOR EXAMINATION OF PULLORUM REACTORS

J. E. Williams
Animal Disease and Parasite Research Branch
Agricultural Research Service
United States Department of Agriculture
Washington 25, D. C.

The National Poultry Improvement Plan presently provides that "in the event that tests to qualify or requalify flocks reveal reactors the owner or the official State agency shall have the right to request that all reactors if 5 or less or 20 percent of all reactors with a minimum of 5 and a maximum of 20 be submitted to a laboratory designated by the official State agency for autopsy and bacteriological examination." The task of culturing reactors is in most cases the responsibility of the State diagnostic laboratory. Minimum recommended culture procedures are printed in the Appendix Sections of the National Poultry Improvement Plan (USDA Miscellaneous Publication 300) and the National Turkey Improvement Plan (USDA Miscellaneous Publication 555).

Procedures currently employed are found to vary to a certain extent with the preferences and experience of poultry pathologists in the various laboratories. This is exemplified by the results of a recent survey among twenty-six laboratories representative of the Northeastern Conference. Similar conditions were found to exist in a recent comparative study of culture methods by the North Central States laboratories. While variations in media used and preparation of inocula were evident, the techniques used were in general aspects comparable. Especially consistent were the organs selected for culture and the techniques used in the final identification of the organisms isolated.

There are some who feel that we should take a new look at current procedures and try as far as possible to resolve the techniques used by the various laboratories into a more standard technique which has the endorsement of all. Since 1951 the Research Committee of the North Central States Poultry Disease Conference has conducted a comparative study of various culture media and techniques for the recovery of Salmonella from reactor birds. The results of this study were published in the 1952 and 1953 Proceedings of the Conference. At the 1954 Conference the results of the survey were summarized and recommendations were made. This material will be published in the 1954 Proceedings.

The fact that more and more attention is being given to organized efforts for the control of paratyphoid infections of fowl requires that we carefully consider our current procedures to insure that they are adequate for detecting all Salmonella types. The mounting evidence of the pathogenicity of the paracolon organisms, especially those members of the Arizona group, so closely related to the Salmonella, requires that we be familiar with the characteristics of the paracolon group and assure ourselves that our current techniques are capable of identifying them. Since organisms other than the Salmonella or paracolons may be isolated from birds reacting to the test, techniques

should insure that these other organisms will be detected. Culture procedures should be adequate for the examination not only of chickens, but also of other fowl. For example, in culturing turkeys more extensive procedures for the bacteriological examination of the intestinal tract should be included.

It is recognized by most laboratories that the main value of culture is in the examination of suspicious reactors, i.e. birds which reveal a low titer to the agglutination test or a suspicious type of reaction. The necessity of routinely culturing typical 4+ reactors is often questioned. There are, of course, instances where the culture of 4+ reactors is desirable as, for example, in the differentiation of S. pullorum and S. gallinarum infections and in those cases where it is desired to gain information regarding the antigenic form type of S. pullorum infection present in a particular flock.

Flock owners should be encouraged to submit birds to the laboratory as soon after testing as possible, and birds should be submitted alive rather than frozen or in some other form. When the birds are received by the laboratory they should be retested with both tube and rapid whole-blood methods, using both standard and polyvalent types of antigen.

The recommendations which follow are, in many respects, identical with those made by the Research Committee of the North Central States Poultry Disease Conference at the meeting this year. These recommendations also follow quite closely the procedures outlined in detail in Biological Products Memo. 54-1 for the isolation and identification of Salmonella organisms from live-virus vaccines of chick-embryo origin. A copy of this Memo. may be secured from the Biologicals Control Section, Animal Disease Eradication Branch, Agricultural Research Service, United States Department of Agriculture, Washington 25, D. C.

In devising any standard procedures for culture foremost consideration should be given to the following items:

(A) Organs for culture.
(B) Collection and preparation of inocula.
(C) Size of inocula.
(D) Initial culture media.
(E) Media for transfer.

Organs for Culture - Present agreement on this item is quite good. If we consider the results of the survey of the Northeastern States and the recommendations of the North Central States, we arrive at the conclusion that the sites most frequently selected for culture include the following: gonads, liver, gall bladder, spleen, heart, pericardium, pancreas, and intestinal tract as well as any other parts revealing lesions.

Collection and Preparation of Inocula - Organs are aseptically collected in toto or in part combined and ground in a sterile blender cup (such as Waring Blendor) for three or four minutes. Nutrient broth or saline may be added to facilitate pipetting.

Intestinal cultures are made directly from the tract into selective media.

Size of Inocula - For inoculation of each 100 ml. of enrichment or nutrient broth a 10 ml. sample of the organ suspension are collected with a sterile wide-mouthed pipette.

Intestinal cultures are made from the cecal tonsils and other portions of the intestinal tract as seems advisable to the pathologist. An inoculum of 1-2 ml. of fecal suspension is placed into each 10-15 ml. of selective broth.

Initial Culture Media - In selecting the initial culture media it must be remembered that all Salmonella strains will not grow equally well on all media. For this reason it is desirable to use at least two and preferably three broths in making initial cultures. For this purpose selenite F and/or tetrathionate broths and a noninhibitory broth such as tryptose broth are recommended. These broths can be dispensed in 100 ml. amounts into 300 ml. erylenmeyer flasks.

If desired one plate each of MacConkey, SS, and tryptose agar may be streaked from the original organ suspension.

Intestinal cultures should be made into either selenite F or tetrathionate broth tubed in 10-15 ml. amounts.

All media are incubated at 37° C. for a period not to exceed 24 hours.

Media for Transfer - In plating the broths, the tetrathionate broth may be plated on one SS plate, the selenite F broth on one MacConkey plate, and the tryptose broth on one plate each of SS agar, MacConkey agar, and tryptose agar.

The selenite F intestinal cultures are streaked on MacConkey agar and the tetrathionate cultures are streaked on SS agar.

All plates are incubated at 37° C. for a period not to exceed 24 hours.

Suspicious colonies from any of the plates are transferred to triple sugar iron slants and subsequently identified by established methods including carbohydrate broths, semisolid medium (motility), indole test medium, urease test medium, and Gram's stain.

Typing of Isolated Cultures - All strains of Salmonella and paracolons isolated should be typed serologically and up-to-date records maintained by the laboratory relative to the types isolated and their distribution. The XII_2 and XII_3 antigenic balance of all strains of S. pullorum isolated should be determined.

Efforts should be made to determine the antigenic relationship to the Salmonella group of any non-Salmonella organisms which may be isolated and thought to be responsible for cross reactions to the agglutination test.

The procedures which have been outlined herein are rather extensive and may be considered by some to exceed requirements. They are presented in the interest of standardization and represent procedures which have been found effective in the recovery of Salmonella as well as other related and non-related types. A host of media for recovery of Salmonella are currently available and media other than those presented in this outline may be preferred by some laboratories.